PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

(Chapter II of the Patent Cooperation Treaty)

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference NKM001PCT	**FOR FURTHER ACTION**	See Form PCT/IPEA/416
International application No. PCT/JP2004/011401	International filing date *(day/month/year)* 02.08.2004	Priority date *(day/month/year)* 01.08.2003
International Patent Classification (IPC) or national classification and IPC C12N5/06		
Applicant NAKANURA, Norimasa et al.		

1. This report is the international preliminary examination report, established by this International Preliminary Examining Authority under Article 35 and transmitted to the applicant according to Article 36.
2. This REPORT consists of a total of 8 sheets, including this cover sheet.
3. This report is also accompanied by ANNEXES, comprising:
   a. ☒ *sent to the applicant and to the International Bureau)* a total of 5 sheets, as follows:
      ☒ sheets of the description, claims and/or drawings which have been amended and are the basis of this report and/or sheets containing rectifications authorized by this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions).
      ☐ sheets which supersede earlier sheets, but which this Authority considers contain an amendment that goes beyond the disclosure in the international application as filed, as indicated in item 4 of Box No. I and the Supplemental Box.
   b. ☐ *(sent to the International Bureau only)* a total of (indicate type and number of electronic carrier(s)) , containing a sequence listing and/or tables related thereto, in computer readable form only, as indicated in the Supplemental Box Relating to Sequence Listing (see Section 802 of the Administrative Instructions).

4. This report contains indications relating to the following items:

☒	Box No. I	Basis of the opinion
☒	Box No. II	Priority
☒	Box No. III	Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
☐	Box No. IV	Lack of unity of invention
☒	Box No. V	Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
☐	Box No. VI	Certain documents cited
☐	Box No. VII	Certain defects in the international application
☐	Box No. VIII	Certain observations on the international application

Date of submission of the demand	Date of completion of this report
20.05.2005	05.10.2005
Name and mailing address of the international preliminary examining authority: European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Authorized Officer Chavanne, F Telephone No. +49 89 2399-8399

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

International application No.
PCT/JP2004/011401

Box No. I Basis of the report

1. With regard to the **language**, this report is based on the international application in the language in which it was filed, unless otherwise indicated under this item.

   ☐ This report is based on translations from the original language into the following language , which is the language of a translation furnished for the purposes of:
   - ☐ international search (under Rules 12.3 and 23.1(b))
   - ☐ publication of the international application (under Rule 12.4)
   - ☐ international preliminary examination (under Rules 55.2 and/or 55.3)

2. With regard to the **elements*** of the international application, this report is based on *(replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report)*:

Description, Pages

1-179	as originally filed

Sequence listings part of the description, Pages

1-206	as originally filed

Claims, Numbers

16, 18-33, 36-109, 111-141, 143-160	as originally filed
1-5, 8-12, 15, 34, 110, 142	received on 20.05.2005 with letter of 20.05.2005

Drawings, Sheets

1/46-46/46	as originally filed

☒ a sequence listing and/or any related table(s) - see Supplemental Box Relating to Sequence Listing

3. ☒ The amendments have resulted in the cancellation of:
   - ☐ the description, pages
   - ☒ the claims, Nos. 6,7,13,14,17,35
   - ☐ the drawings, sheets/figs
   - ☐ the sequence listing *(specify)*:
   - ☐ any table(s) related to sequence listing *(specify)*:

4. ☐ This report has been established as if (some of) the amendments annexed to this report and listed below had not been made, since they have been considered to go beyond the disclosure as filed, as indicated in the Supplemental Box (Rule 70.2(c)).
   - ☐ the description, pages
   - ☐ the claims, Nos.
   - ☐ the drawings, sheets/figs
   - ☐ the sequence listing *(specify)*:
   - ☐ any table(s) related to sequence listing *(specify)*:

* *If item 4 applies, some or all of these sheets may be marked "superseded."*

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

International application No.
PCT/JP2004/011401

Box No. II Priority

1. ☒ This report has been established as if no priority had been claimed due to the failure to furnish within the prescribed time limit the requested:
   - ☒ copy of the earlier application whose priority has been claimed (Rule 66.7(a)).
   - ☐ translation of the earlier application whose priority has been claimed (Rule 66.7(b)).

2. ☐ This report has been established as if no priority had been claimed due to the fact that the priority claim has been found invalid (Rule 64.1). Thus for the purposes of this report, the international filing date indicated above is considered to be the relevant date.

3. Additional observations, if necessary:

Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

1. The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non-obvious), or to be industrially applicable have not been examined in respect of:

   ☐ the entire international application,

   ☒ claims Nos. 97-141

   because:

   ☒ the said international application, or the said claims Nos. 97-141 relate to the following subject matter which does not require an international preliminary examination (specify):

   see separate sheet

   ☐ the description, claims or drawings *(indicate particular elements below)* or said claims Nos. are so unclear that no meaningful opinion could be formed *(specify)*:

   ☐ the claims, or said claims Nos. are so inadequately supported by the description that no meaningful opinion could be formed.

   ☐ no international search report has been established for the said claims Nos.

   ☐ the nucleotide and/or amino acid sequence listing does not comply with the standard provided for in Annex C of the Administrative Instructions in that:

the written form	☐	has not been furnished
	☐	does not comply with the standard
the computer readable form	☐	has not been furnished
	☐	does not comply with the standard

   ☐ the tables related to the nucleotide and/or amino acid sequence listing, if in computer readable form only, do not comply with the technical requirements provided for in Annex C-*bis* of the Administrative Instructions.

   ☐ See separate sheet for further details

Box No. V Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	
	No:	Claims	1-5, 8-12, 15, 16, 18-34, 36-160
Inventive step (IS)	Yes:	Claims	
	No:	Claims	1-5, 8-12, 15, 16, 18-34, 36-160
Industrial applicability (IA)	Yes:	Claims	1-5, 8-12, 15, 16, 18-34, 36-96,141-160
	No:	Claims	

2. Citations and explanations (Rule 70.7):

see separate sheet

Supplemental Box relating to Sequence Listing

Continuation of Box I, item 2:

1. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application and necessary to the claimed invention, this report has been established on the basis of:

   a. type of material:

   ☒ a sequence listing

   ☐ table(s) related to the sequence listing

   b. format of material:

   ☒ in written format

   ☒ in computer readable form

   c. time of filing/furnishing:

   ☒ contained in the international application as filed

   ☒ filed together with the international application in computer readable form

   ☐ furnished subsequently to this Authority for the purposes of search and/or examination

   ☐ received by this Authority as an amendment on

2. ☐ In addition, in the case that more than one version or copy of a sequence listing and/or table(s) relating thereto has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

3. Additional observations, if necessary:

III. Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

1. Since claims 97-141 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition. In consequence to this incomplete search, an opinion with regard to novelty, inventive step and industrial applicability can only be partially formulated on the basis of the searched subject-matter of these claims.

V. Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Reference is made to the following documents:

   D1: Nature biotechnology
   vol. 18, pp. 954-958, 2000
   D2: WO 95/30742
   D3: Journal of Biomedical Materials Research
   Vol. 45, No. 5, pp. 355-362, 1999
   D4: WO 95/33821
   D5: US 2003/091979

2. D1 describes the production of a three dimensional synthetic human bone by culturing osteogenic cells in a culture media containing the ECM synthesis promoting agent TGF-beta. The cells secrete an extracellular matrix containing several proteins, among others collagen I and III (abstract; page 954, column 2; page 955, column 1; page 956, column 2).
   Thus, in view of D1, the subject-matter of claims 71-85, 88, 92-96 and 149-160 is not novel (Article 33(2) PCT).
   D1 does not give an exhaustive list of the components of the extracellular matrix it discloses. D1 mentions that said extracellular matrix comprises several proteins. Fibronectin is not specifically mentioned in D1. However, since fibronectin is a regular extracellular matrix component, it cannot be excluded that fibronectin is comprised in

the extracellular matrix of D1. Hence, in the absence of any evidence of the contrary, the subject-matter of claims 1-33 cannot be considered novel (Article 33(2) PCT).

3. D2 describes a composition comprising cells and the extracellular matrix synthesis promoting agents TGF-beta or ascorbic acid. Three dimensional synthetic tissue for cartilage repair is produced using said composition. The resulting tissue is free of scaffold. D2 further shows that the chondrogenic cells used to produce said synthetic tissue secrete collagen I (abstract; pages 20-40; examples 3 and 4). D2 describes, like example 7 of the present demand, chondrogenesis induction using TGF-beta to culture a synthetic tissue. Thus, in view of D2, the subject-matter of claims 1-33, 71-86, 88-112, 114-141 and 149-160 is not novel (Article 33(2) PCT).

4. D3 describes a synthetic tissue composed of a monolayer cell sheet without a scaffold which secretes fibronectin in reaction to a temperature stimulus (abstract; page 355, column 2 to page 356, column 2, paragraph 2; page 357, column 2, paragraph 2; page 359 to page 361, column 1, paragraph 1). Thus, in view of D3, the subject-matter of claims 34-36, 42, 43, 48, 51-53, 62-64, 74-79 and 81-85 is not novel (Article 33(2) PCT).

5. D4 describes a three dimensional synthetic tissue made of cells and extracellular matrix secreted by these cells. The culture medium of the cells comprises an extracellular matrix synthesis promoting agent such as TGF-beta or ascorbic acid. D4 mentions the use of a physical stimulus (stretching) to increase cell division (abstract; pages 15-30; pages 45 and 46). Thus, in view of D4, the subject-matter of claims 34-41, 43-86, 88-112 and 114-160 is not novel (Article 33(2) PCT).

6. D5 describes a three dimensional muscle tissue such as a heart. D5 mentions physical and electrical stimuli for the production of said tissue (abstract; paragraphs 1, 8, 14, 16, 31, 83-85, 112, example 2). Thus, in view of D5, the subject-matter of claims 74-77, 85-87, 91-94, 96-149, 150, 154, 156 and 157 is not novel (Article 33(2) PCT).

7. For the assessment of the present claims 97-141 on the question whether they are industrially applicable, no unified criteria exist in the PCT Contracting States. The

patentability can also be dependent upon the formulation of the claims. The EPO, for example, does not recognize as industrially applicable the subject-matter of claims to the use of a compound in medical treatment, but may allow, however, claims to a known compound for first use in medical treatment and the use of such a compound for the manufacture of a medicament for a new medical treatment.

CLAIMS

1. An implantable synthetic tissue, which is substantially made of cells and an extracellular matrix (ECM) derived from the cells, and is free of scaffolds, said extracellular matrix compirses fibronectin, wherein the extracellular matrix is diffusedly distributed in the tissue, and wherein the extracellular matrix and the cells are integrated together into a three-dimensional structure.

2. A synthetic tissue according to claim 1, which is biologically organized in the third dimensional direction.

3. A synthetic tissue according to claim 1, which has biological integration capability with surroundings.

4. A synthetic tissue according to claim 3, wherein the biological integration capability includes capability to adhere to surrounding cells and/or extracellular matrices.

5. A synthetic tissue according to claim 1, which comprises cells.

6. (Canceled)

7. (Canceled)

8. A synthetic tissue according to claim 1[7], wherein the extracellular matrix further contains at least one selected from the group consisting of collagen I, collagen III, and vitronectin[and fibronectin].

9. A synthetic tissue according to claim 1[7], wherein the extracellular matrix contains collagen I, collagen III, vitronectin and fibronectin.

10. A synthetic tissue according to claim 1[7], wherein the extracellular matrix contains vitronectin.

11. A synthetic tissue according to claim 1[7], wherein the extracellular matrix contains fibronectin.

12. A synthetic tissue according to claim 1[7], wherein the extracellular matrix contains collagen I and collagen III, the collagen constitutes 5% to 25% of the tissue, and the ratio of the collagen I to the collagen III is between 1:10 and 10:1.

13. (Canceled)

14. (Canceled)

15. A synthetic tissue according to claim 1, wherein [an extracellular matrix is diffusedly distributed, and] the distribution densities of the extracellular matrix in two arbitrary sections of 1 cm^2 in the tissue have a ratio within a range of about 1:3 to about 3:1.

16. A synthetic tissue according to claim 1 which is heterologous, allogenic, isologous, or autogenous.

17. (Canceled)

34. A method for producing a synthetic tissue, comprising the steps of:

A) providing cells;

B) placing the cells in a container, the container having cell culture medium containing an ECM synthesis promoting agent and having a sufficient base area which can accommodate a synthetic tissue having a desired size;

C) culturing the cells in the container along with the cell culture medium containing the ECM synthesis promoting agent for a period of time sufficient for formation of the synthetic tissue having the desired size; and

D) detaching the cells from the container, wherein a stimulus for inducing tissue contraction is applied in the detaching step.

35. (canceled)

36. A method according to claim 35, wherein the stimulus includes a physical or chemical stimulus.

37. A method according to claim 36, wherein the physical stimulus includes shaking of the container, pipetting, or deformation of the container.

38. A method according to claim 34, wherein the detaching step includes adding an actin regulatory agent.

39. A method according to claim 38, wherein the actin regulatory agent includes a chemical substance selected from the group consisting of actin depolymerizing agents and actin polymerizing agents.

40. A method according to claim 39, wherein the actin depolymerizing agent is selected from the group consisting

107. A method according to claim 97, wherein an extracellular matrix is provided on a surface of the complex.

108. A method according to claim 97, wherein an extracellular matrix is diffusedly distributed on a surface of the complex.

109. A method according to claim 97, wherein an extracellular matrix is diffusedly distributed on a surface of the complex, and the distribution densities of the extracellular matrix in two arbitrary sections of 1 cm^2 have a ratio within a range of about 1:3 to about 3:1.

110. A method according to claim 97, wherein an extracellular matrix is diffusedly distributed on a surface of the complex, and the distribution densities of the extracellular matrix in two arbitrary sections of 1 cm^2 have a ratio within a range of about 1:2 to about 2:1.

111. A method according to claim 97, which is heterologous, allogenic, isologous, or autogenous.

112. A method according to claim 97, wherein the portion includes a bag-shaped organ.

113. A method according to claim 112, wherein the bag-shaped organ includes a heart.

114. A method according to claim 97, wherein the complex resists the expansion and contraction of the portion.

115. A method according to claim 97, wherein the complex has biological integration.

140. A method according to claim 133, which is substantially made of cells and an extracellular matrix derived from the cells, wherein the other synthetic tissue includes an artificial bone or a microfibrous collagen medical device.

141. A method according to claim 139, the artificial bone includes hydroxyapatite.

142. A method for producing a synthetic tissue, comprising the steps of:

A) providing cells;

B) placing the cells in a container, the container having cell culture medium containing an ECM synthesis promoting agent and having a sufficient base area which can accommodate a synthetic tissue having a desired size;

C) culturing the cells in the container along with the cell culture medium containing the ECM synthesis promoting agent for a period of time sufficient for formation of the synthetic tissue having the desired size; and

D) regulating a thickness of the synthetic tissue by a physical or chemical stimulus to a desired thickness.

143. A method according to claim 142, wherein the physical stimulus includes shear stress between the synthetic tissue and the container, deformation of the base of the container, shaking of the container, or pipetting.

144. A method according to claim 142, wherein the chemical stimulus is obtained by using a chemical substance selected from the group consisting of actin depolymerizing agents and actin polymerizing agents.